大鼠灌胃多糖铁复合物后尿液蛋白质组的变化

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摘要：铁是维持生物体正常生理功能所必需的微量元素，还没有研究从尿液蛋白质组的角度探究铁元素对机体的整体影响。本研究对大鼠灌胃多糖铁复合物（28mg/kg·d 铁元素，相当于成年人预防贫血的剂量）4 天，采用自身前后比较和成组比较两种分析方法，对比分析了大鼠短期灌胃多糖铁复合物前后的尿液蛋白质组。许多差异蛋白被报道与铁有关，包括 2'，3'-环核苷酸 3'-磷酸二酯酶(CNPase)（灌胃前是灌胃后的 7.7 倍，p=0.0039）、p38（灌胃后是灌胃前的 14.5 倍，p=0.003）等；单只大鼠前后比较中，铁调素（Hepcidin）在 4 只大鼠中同时上调。差异蛋白富集到的生物学过程包括对碳水化合物代谢过程、铁离子的反应、细胞凋亡过程的调控、造血祖细胞分化等；分子功能（如补体结合、血红蛋白结合等）、KEGG 通路（如补体和凝血级联、胆固醇代谢、疟疾等）也显示出与铁的相关性。本研究从尿液蛋白质组学的角度有助于深入理解铁元素的生物学功能，并为铁代谢紊乱相关疾病的预防、诊断、治疗及监测提供了新的研究视角。

关键词：铁；尿液；蛋白质组；多糖铁复合物；营养素；矿物质元素。

Changes of urine proteome after intragastric administration of polysaccharide iron complex in rats

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Abstract: Iron is an essential trace element to maintain the normal physiological function of organisms. No studies have investigated the overall effect of iron on the body from the perspective of urine proteome. In this study, the urine proteome of rats before and after short-term intragastric administration of polysaccharide-iron complex (28mg/kg·d iron, which is equivalent to the dose of anemia prevention in adults) was compared and analyzed by using two analysis methods: individual comparison and group comparison. Many different proteins were reported to be related to iron, including 2', 3' -cyclic nucleotide 3' -phosphodiesterase (CNPase) (7.7 times higher than that after gavage, p=0.0039), p38 (14.5 times higher than that before gavage, p=0.003), etc. In the individual comparison, Hepcidin was up-regulated in 4 rats simultaneously. The biological processes of differential protein enrichment include carbohydrate metabolism, iron ion reaction, apoptosis regulation, hematopoietic progenitor cell differentiation, etc. Molecular functions (e.g., complement binding, hemoglobin binding, etc.), KEGG pathways (e.g., complement and coagulation cascade, cholesterol metabolism, malaria, etc.) have also been shown to be associated with iron. This study contributes to the in-depth understanding of the biological function of iron from the perspective of urine proteomics, and provides a new research perspective for the prevention, diagnosis, treatment and monitoring of iron-related disorders.

Key words: Iron; Urine; Proteome; Polysaccharide-iron complex; Nutrients; Mineral elements.

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1 引言

微量元素在机体的各种生理过程中扮演着不可或缺的角色。近年来，随着对于微量元素作用的深入研究，科学家们逐渐认识到微量元素的稳态也与多种疾病的发病机制有关。

铁是维持生物体正常生理功能所必需的微量元素之一，它参与了体内多种重要生物过程，比如，氧的运输、细胞呼吸以及 DNA 合成等。铁代谢紊乱可能导致机体内生化平衡失调，引发一系列健康问题[1]。

由于尿液不属于内环境，对比血浆，尿液不存在稳态的机制，能够积累机体生理状态的早期变化，更敏感地反映出机体变化情况，是下一代生物标志物的来源[2]。尿液中的蛋白质包含丰富的信息，可以反映出机体不同系统、不同器官产生的微小变化。

本实验室之前报道过，尿液蛋白质组能够较为系统、全面地反映苏糖酸镁摄入对机体产生的影响，有潜力为临床营养学研究和实践提供线索[3]。但是至今为止，还没有从尿液蛋白质组的角度探究铁元素对机体的影响的研究。

本研究选择了多糖铁复合物（Polysaccharide-Iron Complex）作为铁元素补充剂，可迅速提高血铁水平与升高血红蛋白。对于胃肠黏膜刺激性轻，不良反应小，可连续给药，吸收率较高，用于防治缺铁性贫血。本研究旨在探究大鼠短期摄入多糖铁复合物后尿液蛋白质组的变化，以进一步了解铁元素在生物体内的生物学功能及其整体影响，为营养学研究提供新的研究视角。

2 材料与方法

2.1 实验材料

2.1.1 实验耗材

5ml 无菌注射器（BD 公司）、灌胃针（16 号，80mm，弯针）、1.5ml/2ml 离心管（美国 Axygen 公司）、50ml/15ml 离心管（美国 Corning 公司）、96 孔细胞培养板（美国 Corning 公司）、10kD 滤器（美国 Pall 公司）、Oasis HLB 固相萃取柱（美国 Waters 公司）、1ml/200ul/20ul 移液枪头(美国 Axygen 公司)、BCA 试剂盒(美国 Thermo Fisher Scientific 公司)、高 pH 反向肽分离试剂盒（美国 Thermo Fisher Scientific 公司）、iRT (indexed retention time, 英国 BioGnosis 公司)。

2.1.2 实验仪器

大鼠代谢笼（北京佳源兴业科技有限公司）、冷冻高速离心机（美国 Thermo Fisher Scientific 公司）、真空浓缩仪（美国 Thermo Fisher Scientific 公司）、DK-S22 电热恒温水浴锅（上海精宏实验设备有限公司）、全波长多功能酶标仪（德国 BMG Labtech 公司）、振荡器（美国 Thermo Fisher Scientific 公司）、TS100 恒温混匀仪（杭州瑞诚仪器有限公司）、电子天平（瑞士 METTLER TOLEDO 公司）、-80℃超低温冷冻冰箱（美国 Thermo Fisher Scientific 公司）、EASY-nLC1200 超高效液相色谱（美国 Thermo Fisher Scientific 公司）、Orbitrap Fusion Lumos Tribird 质谱仪（美国 Thermo Fisher Scientific 公司）。

2.1.3 实验试剂

多糖铁复合物胶囊（国药准字 H20030033）由上海医药集团青岛国风药业分股份有限公司生产。此外，还使用了胰酶 Trypsin Golden（美国 Promega 公司）、二硫苏糖醇 DTT（德国 Sigma 公司）、碘乙酰胺 IAA（德国 Sigma 公司）、碳酸氢铵 NH4HCO3（德国 Sigma 公司）、尿素 Urea（德国 Sigma 公司）、纯净水（中国娃哈哈公司）、质谱级甲醇（美国 Thermo Fisher Scientific 公司）、质谱级乙腈（美国 Thermo Fisher Scientific 公司）、质谱级纯水（美国 Thermo Fisher Scientific 公司）、Tris-Base（美国 Promega 公司）、硫脲 Throurea（德国 Sigma 公司）等试剂。

2.1.4 分析软件

Proteome Discoverer(Version2.1,美国 Thermo Fisher Scientific 公司)、Spectronaut

Pulsar（英国 Biognosys 公司）、Ingenuity Pathway Analysis（德国 Qiagen 公司）；R studio（Version1.2.5001）；Xftp 7；Xshell 7。

2.2 实验方法

2.2.1 动物模型建立

本研究使用 17 周龄大鼠进行研究，尽量减少灌胃期间生长发育带来的影响。健康 SD（Sprague Dawley）9 周龄雄性大鼠（250±20g）5 只，购于北京维通利华实验动物技术有限公司。大鼠在标准环境中（室温（22±2）℃，湿度 65%-70%）饲养 8 周后，体重达到 500-600g，开始实验，一切实验操作遵循北京师范大学生命科学学院伦理委员会的审查和批准。

膳食营养素的可耐受最高摄入量(UL，tolerable upper intake levels)：指某一生理阶段和性别人群，几乎对所有个体健康都无任何副作用和危险的平均每日营养素最高摄入量。推荐摄入量(recommended nutrient intakes, RNI)，指可满足某一特定年龄、性别、生理状况群体 97-98%个体需要量的摄入水平。

根据中国居民膳食指南，铁的每日推荐摄入量（RNI）为 20mg/d，可耐受最高摄入量(UL)为 42mg/d[4]，按照多糖铁复合物说明书指示的预防贫血剂量，多糖铁复合物胶囊每粒含有铁元素 150mg，成人每日吃 1-2 粒，即摄入铁 150-300mg/d，此剂量按照体表面积和体重换算成大鼠的铁剂量约等于 14-28mg/kg·d。本研究中，大鼠灌胃铁的剂量为 28mg/kg·d，相当于成年人预防贫血的剂量。将 3g 多糖铁复合物（按铁含量计约为 1.4g）溶解于 500ml 无菌水中，配置成灌胃溶液。每只大鼠每天灌胃 5ml 多糖铁溶液，每天灌胃 1 次，连续灌胃 4 天。灌胃第一天记为 Fe-D1，以此类推。在灌胃前和灌胃后分别设置取样时间点，进行自身前后对照，灌胃前一天收集的样本为对照组，记为 Fe-D0，样本编号为 51-55。灌胃第 4 天收集的样本为实验组，记为 Fe-D4 样本编号为 61-65。

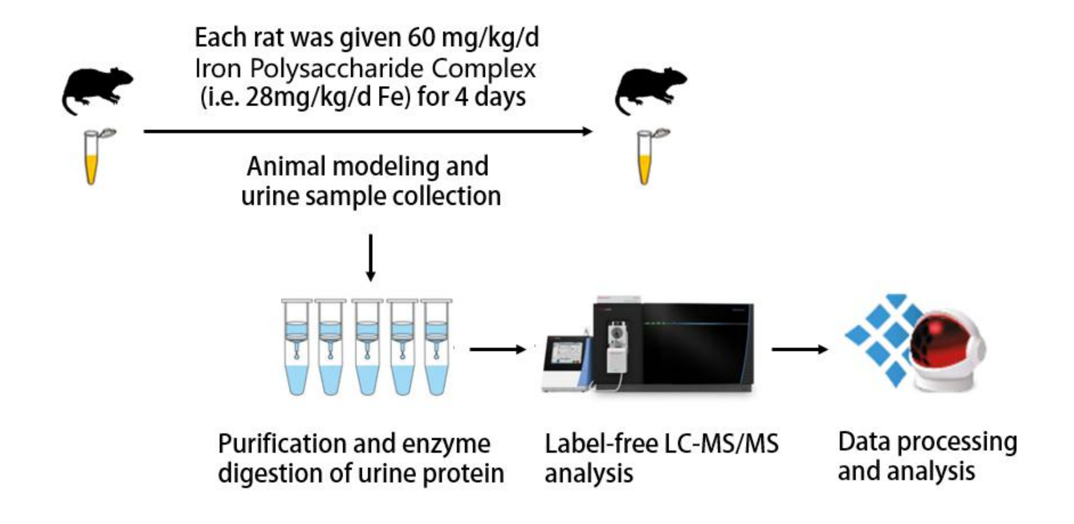


图 1 研究方法与技术路线

2.2.2 尿液样本收集

在开始灌胃多糖铁复合物前一天（D0）和灌胃多糖铁复合物 4 天后(D4)，将每只大鼠在同一时间单独放入代谢笼中，禁食禁水 12h，过夜收取尿液，尿液样本收集后置于-80℃冰箱暂存备用。

2.2.3 尿液样本处理

取出 2ml 尿样解冻，4℃，12000×g 条件下离心 30 分钟，去除细胞碎片，取上清液加入 1M 二硫苏糖醇（Dithiothreitol, DTT, Sigma）贮液 40ul，达到 DTT 的工作浓度 20mM，混匀后金属浴 37℃加热 60 分钟，晾凉至室温后，加入碘乙酰胺(Iodoacetamide, IAA, Sigma)

贮液100ul，达到IAM的工作浓度，混匀后常温避光反应45分钟。反应结束后，将样本转移至新的离心管中，与三倍体积的预冷无水乙醇充分混合，置于-20℃冰箱中24小时沉淀蛋白。沉淀结束，4℃，10000×g 条件下离心 30 分钟，弃去上清，干燥蛋白沉淀，向蛋白沉淀中加入200ul 20mM Tris溶液复溶。复溶后的样品离心后保留上清液，采用 Bradford 法测定蛋白质浓度。使用滤器辅助样品制备（FASP）的方法，将尿蛋白提取液加入 10kD 超滤管（Pall, Port Washington, NY, USA）的滤膜上，分别加入20mM Tris溶液洗涤三次，加入30mM Tris溶液重溶蛋白，每个样品按比例（尿蛋白：胰酶=50：1）加入胰蛋白酶（Trypsin Gold, Mass Spec Grade, Promega, Fitchburg, WI, USA）进行消化，37℃孵育16小时，酶解后的滤液即为多肽混合液。收集到的多肽混合液通过Oasis HLB固相萃取柱进行除盐处理后真空干燥，置于-80℃保存。加入30微升0.1%甲酸水将冻干多肽粉末复溶后，使用 BCA 试剂盒对肽段浓度进行测定，将肽段浓度稀释至0.5μg/μL，每个样本取出4微升作为mix样本。

2.2.4 LC-MS/MS 串联质谱分析

所有鉴定样品以样品∶iRT为20∶1 的体积比例加入稀释100倍的iRT标准液，统一保留时间。对所有样本进行数据非依赖性采集（DIA），每个样本重复3次，每隔10针插入1针mix样本作为质量控制。将1ug样本使用EASY-nLC1200液相色谱分离(洗脱时间：90min，梯度:流动相A:0.1%甲酸、流动相B:80%乙腈),洗脱下来的肽段进入Orbitrap Fusion Lumos Tribird质谱仪分析，生成样品对应的raw文件。

2.2.5 数据处理和分析

将DIA模式下采集的raw文件导入Spectronaut软件分析，高度可信蛋白标准为肽段q value<0.01，应用峰面积定量法对二级肽段所有碎片离子峰面积进行蛋白定量，自动归一化处理。

保留含有两个或以上特异肽段的蛋白，将缺失值替换成0，计算各个样本鉴定到的不同蛋白含量，将大鼠灌胃多糖铁复合物前的样本与灌胃多糖铁复合物4天后的样本进行比较，筛选差异蛋白。

利用悟空平台(https://omicsolution.org/wkomics/main/)进行非监督聚类分析(HCA)、主成分分析（PCA）、OPLS-DA分析。使用DAVID数据库（https://david.ncifcrf.gov/）进行差异蛋白功能富集分析，得到生物学过程、细胞定位和分子功能3个方面的结果。基于Pubmed数据库（https://pubmed.ncbi.nlm.nih.gov/）对差异蛋白和相关通路进行搜索。使用STRING数据库进行蛋白互作网络分析（https://cn.string-db.org/)。

2.2.6 随机分组分析

在使用组学技术研究疾病生物标志物时，通常筛选疾病组与对照组之间的差异。由于组学数据庞大而样本量有限，两组之间的差异可能是随机产生的。为此，我们使用随机分组统计分析策略，该策略适用于样本量有限的临床组学疾病生物标志物的研究，并确定两组之间的差异是否随机产生[5]。

将灌胃前（n=5）和灌胃后（n=5）共10个样本随机分成两组，在所有随机组合中，按照相同的的筛选条件计算所有随机组合的差异蛋白数目的平均值。

2.2.7 利用Pubmed数据库对于差异蛋白和功能注释进行分析

在Pubmed对于差异蛋白和功能注释进行搜索和分析，具体搜索条件是在标题或摘要中同时包含关键词和铁，例如，“iron[Title/Abstract] AND heme [Title/Abstract]”。然后再逐一对这些文章进行阅读和筛选，分析差异蛋白以及差异蛋白富集到的分子功能、生物学过程、通路等与铁的关联。

3 结果与讨论

3.1 灌胃多糖铁复合物后大鼠的特征

在实验过程中，我们观察了大鼠在灌胃多糖铁复合物后的饮水、进食、体重、毛发等特征。发现灌胃多糖铁复合物前后，大鼠体重基本保持稳定，饮水、进食、活动正常。灌胃多糖铁复合物后，大鼠的粪便颜色漆黑，毛发较为杂乱，可能是铁摄入过多所致。

3.2 尿液蛋白质鉴定情况和非监督聚类分析

灌胃多糖铁复合物前（D0）与灌胃第 4 天（D4）的尿液样本共鉴定到 1803 个蛋白（满足 unique peptides>1，FDR<1%）。对总蛋白进行非监督聚类分析（HCA）和主成分分析（PCA），结果如图 2 和图 3 所示。HCA 和 PCA 的结果显示，摄入多糖铁复合物后大鼠尿液蛋白质组发生了比较显著的变化，这可能反映了机体对于外源性铁的迅速响应。但样本点分布较分散，表明个体间存在一定差异。

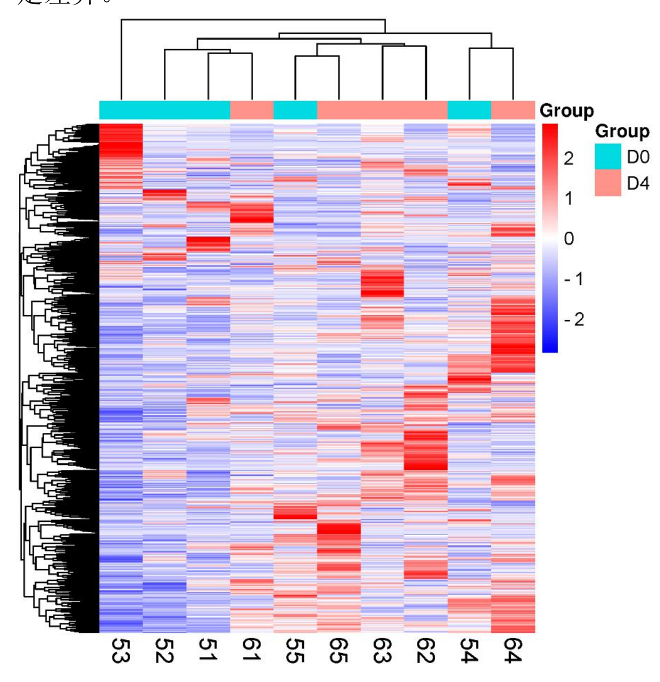


图 2 灌胃多糖铁复合物前与灌胃第 4 天的尿液样本总蛋白非监督聚类分析（HCA）

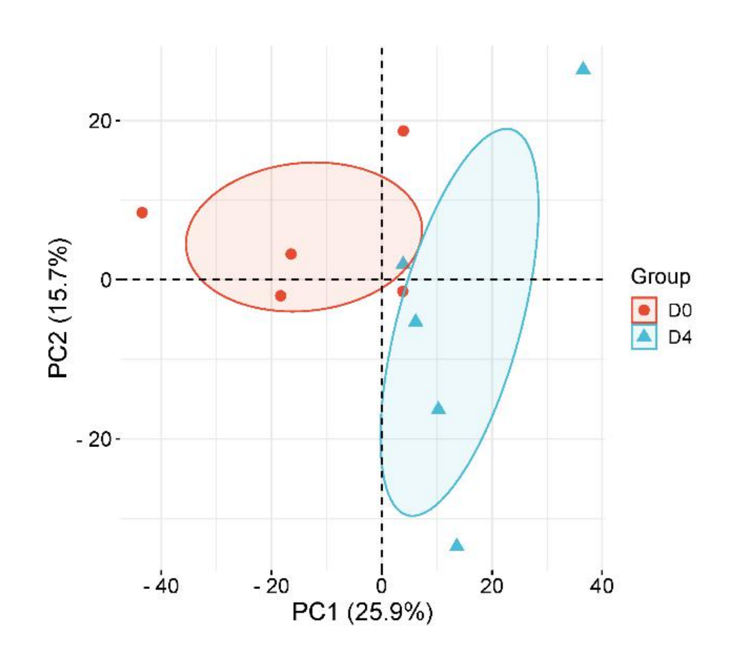


图 3 灌胃多糖铁复合物前与灌胃第 4 天的尿液样本总蛋白主成分分析（PCA）

3.3 成组比较

3.3.1 差异蛋白分析

将缺失值替换成 0，将大鼠灌胃前样本与灌胃第 4 天样本进行成组比较，筛选出 157 个差异蛋白。筛选差异蛋白条件是：T 检验分析 P 值<0.05， Fold change (FC)>1.5 或<0.67。详见补充表格。

其中，有 52 个差异蛋白 P 值<0.01，灌胃前后变化十分显著，如表 1 所示。

利用 PubMed 数据库对 52 个差异蛋白进行蛋白功能的分析和文献检索，将显示差异蛋白与铁相关性的文献列在表格中。

大鼠摄入多糖铁后，尿液中下调的蛋白质包括 2’，3’-环核苷酸 3’-磷酸二酯酶(2’,3’-cyclic-nucleotide 3’-phosphodiesterase, CNPase)、S100 钙结合蛋白 A7（S100 calcium binding protein A7 like 2,S100A7l2)、金属蛋白酶抑制剂 1 (Tissue inhibitor of metalloproteinases 1, TIMP-1)、整合膜蛋白 2B(Integral membrane protein 2B)。CNPase 是一种髓鞘标志物,FC 为 0.13。S100A7 是一种能够诱导免疫调节活性的蛋白质。在孕期缺铁饮食大鼠的子代大脑中发现，CNPase、S100 钙结合蛋白的表达量降低，说明铁的可用性影响少突胶质细胞的发育[6]。与对照大鼠相比，TIMP-1 在服用铁螯合剂去铁酮的大鼠中过表达。

原癌基因 c-Crk 衔接分子(p38)的 FC 为 14.53。铁过载骨髓间充质干细胞(BMSC)中 p38 蛋白表达上调[7]。鼻咽癌细胞内胆固醇转运蛋白（NPC intracellular cholesterol transporter 1）的 FC 为 4.38。研究表明，铁超载增加细胞内胆固醇[8]。碳酸酐酶(Carbonic anhydrase, CA)的 FC 为 3.8。对实验动物的研究表明，红细胞中氧化应激升高会导致形成针对碳酸酐酶和贫血的自身抗体[9]；碳酸酐酶可能对铁代谢有干扰作用[10]。

表 1 Fe-D0 组和 Fe-D4 组比较分析中变化显著的差异蛋白（P 值<0.01，FC>1.5 或<0.67）

Protein Accessions	Genes	FC	P	Related to Iron
P13233	Cnp	0.1258	0.0039	[6]
D3Z9U8	S100a7l2	0.3625	0.0026	[6]
P30120	Timp1	0.3759	0.0019	[11]
Q5XIE8	Itm2b	0.4378	0.0066	
A0A0G2JTC1	Lilra5	1.5487	0.0076	
P25236	Selenop	1.5491	0.0051	[12]
Q8CHN3	Wfdc2	1.5622	0.0090	
P97710	Sirpa	1.5810	0.0092	
P10354	Chga	1.5829	0.0051	[13]
Q501W2	Cd27	1.5953	0.0086	[14]
D3ZM39	Dsg1	1.6098	0.0015	
F1LUV9	Ncam1	1.6366	0.0093	
C0JPT7	Flna	1.6394	0.0072	
P50430	Arsb	1.6583	0.0085	
A0A0H2UI19	F12	1.6662	0.0071	[15]
Q99MA2	Xpnpep2	1.6707	0.0043	
Q6P9V1	Cd81	1.6736	0.0090	[16]
E9PSQ1	Amy1a	1.7125	0.0014	
P85971	Pgls	1.7265	0.0089	

P07314	Ggt1	1.7329	0.0029	[17]
Q568Z6	Ist1	1.7486	0.0034	
Q6TUD4	Yipf3	1.7736	0.0007	
A0A0G2K3G0	Hrg	1.7893	0.0006	[18]
D3ZUM4	Glb1	1.8017	0.0097	[19]
A0A096P6L8	Fn1	1.8337	0.0070	[20]
P51635	Akr1a1	1.8442	0.0051	[21]
P10247	Cd74	1.8848	0.0029	[22]
G3V8X5	Slc5a10	1.8860	0.0079	
D4A263	Plekhb2	1.8904	0.0094	
Q9ES53	Ufd1	1.9660	0.0061	
P61459	Pcbd1	1.9943	0.0058	
D4A6I7	Psca	2.0741	0.0002	
Q62894	Ecm1	2.0797	0.0069	
G3V928	Lrp1	2.1202	0.0023	[23]
Q64319	Slc3a1	2.1421	0.0072	[24,25]
P13221	Got1	2.1563	0.0078	[26,27]
F1LLW8	Ids	2.1612	0.0048	
Q68FY0	Uqcrc1	2.1790	0.0055	[28]
G3V6A0	Pdgfra	2.5490	0.0002	
D3ZFC6	Itih4	2.5596	0.0090	
F1LQT4	Cpn2	2.6775	0.0033	
Q5M891	C4bpa	2.6898	0.0055	
D3ZWD6	C8a	2.7839	0.0009	
A0A088DKH8	Amhr2	3.2162	0.0036	
A2IBE2	Ca12	3.8027	0.0088	[9,10]
G3V7K5	Npc1	4.3789	0.0006	[8,29]
A0A0G2K227	Slc6a6	7.1754	0.0021	
Q6AYC4	Capg	7.9503	0.0011	[30]
O70257	Stx7	11.6417	0.0010	
D3ZAT0	Svs3b;Svs3a	12.2337	0.0064	
Q63768	Crk	14.5319	0.0030	[7]
D4A076	Btn2a2	20.8981	0.0005	

3.3.2 随机分组结果

为了确定成组比较鉴定到的差异蛋白随机产生的可能性，我们对两组 10 个样本鉴定到的总蛋白进行了随机分组的验证，应用同样的筛选差异蛋白的标准：FC≥1.5 或≤0.67，P＜0.01，进行了 126 次随机分组得到的差异蛋白平均为 10.82 个，随机鉴定蛋白的比例为 21.15%，表明至少有 79.85%比例的差异蛋白不是由于随机性产生的。随机分组检验的结果见表 2，我们筛选得到的 52 个差异蛋白（FC≥1.5 或≤0.67，P＜0.01）是随机产生的概率很低，结果显示这些差异蛋白确实与多糖铁复合物补剂短期摄入相关。

表 2 按照 FC≥1.5 or ≤0.67，P<0.01 的筛选条件对 Fe-D0 组和 Fe-D4 组进行随机分组结果

Screening criteria	number of differential proteins	Total number of random combinations	Average number of proteins with false random combinations	Ratio(average numbers of proteins with false random combinations/number of correctly identified differential proteins)
FC≥1.5 or ≤ 0.67, P <0.01	52	126	11	21.15%

我们还按照 FC≥1.5 或≤0.67，P<0.05 的筛选条件对两组 10 个样本鉴定到的总蛋白进行了随机分组的验证，进行了 126 次随机分组得到的差异蛋白平均为 55 个，随机鉴定蛋白的比例为 35.08%，表明至少有 65%比例的差异蛋白不是由于随机性产生的。我们筛选得到的 157 个差异蛋白（FC≥1.5 或≤0.67，P<0.05）是随机产生的概率较低。

3.3.3 生物学通路分析

将 157 个差异蛋白（P 值<0.05，FC>1.5 或<0.67）导入 DAVID 数据库，富集到 53 个生物学过程（BP），如表 3 所示。

多个生物学通路被报道与铁的生物学功能有关。如补体激活、葡萄糖跨膜转运、对雌激素的反应、建立内皮屏障、细胞凋亡过程的负调节、对铁离子的反应、碳水化合物代谢过程、酶原活化、细胞对白细胞介素-6 的反应、钠离子传输、细胞基质粘附、造血祖细胞分化等。

根据文献，静脉注射铁制剂诱导体内补体活化[31]。铁代谢失调影响衰老[32]。全身性、细胞性铁和葡萄糖代谢途径是相互关联的[33]。雌激素水平升高与全身可利用的铁增加有关[34]。雌激素给药上调转铁蛋白[35]。长期施用地塞米松的大鼠肝铁浓度降低[36]。细胞内铁的螯合增强内皮屏障功能[37]。铁诱导活性氧（ROS）产生和细胞凋亡[38]。较低的血清铁水平与较高的血清 IL-6 水平显著相关，IL-6 通过诱导 ROS 依赖性脂质过氧化和破坏铁稳态来促进支气管上皮细胞中的铁死亡[39,40]。支气管上皮细胞中铁的蓄积依赖于钠转运[41]。L-抗坏血酸能够促进铁吸收[42]。宿主抗菌机制可降低铁对病原体的可用性，影响先天免疫反应的铁蛋白有多种[43]。铁蛋白对人造血祖细胞的体外生长和体外 T 淋巴细胞的增殖具有抑制作用[44]。铁超载抑制软骨内骨化[45]。铁调节两种类型哺乳动物细胞中 L-胱氨酸的摄取和下游 GSH 的产生[46]。由于篇幅有限，其他生物学过程及其与铁的相关文献见表格。

表 3 Fe-D0 组和 Fe-D4 组差异蛋白（P 值<0.05，FC>1.5 或<0.67）的生物学过程（BP）富集分析（P 值<0.05，按照 P 值从小到大排序）

Term	Count	%	P-Value	Related to Iron
complement activation	5	3.4	0.000025	[47]
aging	12	8.1	0.000041	[32]
glucose transmembrane transport	4	2.7	0.00095	[33]
complement activation, classical pathway	5	3.4	0.0013	[47]
response to estrogen	6	4.1	0.0016	[34,35]
positive regulation of peptidyl-tyrosine phosphorylation	6	4.1	0.0018	
metanephric proximal tubule development	3	2	0.0022	[48]
regulation of cell shape	6	4.1	0.0044	

establishment of endothelial barrier	3	2	0.011	[37]
organic anion transport	3	2	0.011	
negative regulation of apoptotic process	11	7.4	0.014	[38]
transmembrane transport	7	4.7	0.014	
protein stabilization	6	4.1	0.015	
response to iron ion	3	2	0.016	
carbohydrate metabolic process	5	3.4	0.017	[49]
cellular response to dexamethasone stimulus	4	2.7	0.018	[36]
positive regulation of fibroblast proliferation	4	2.7	0.021	[50]
negative regulation of intestinal absorption	2	1.4	0.022	
cellular carbohydrate metabolic process	2	1.4	0.022	[49]
urate salt excretion	2	1.4	0.022	[51]
camera-type eye development	4	2.7	0.023	[52]
neuron migration	5	3.4	0.026	[53]
response to ethanol	6	4.1	0.026	[54,55]
zymogen activation	3	2	0.026	[56]
cell adhesion mediated by integrin	3	2	0.028	[50,57]
defense response to Gram-negative bacterium	4	2.7	0.028	[58]
T cell activation via T cell receptor contact with antigen bound to MHC molecule on antigen presenting cell	2	1.4	0.029	
transepithelial water transport	2	1.4	0.029	
regulation of macrophage migration	2	1.4	0.029	[59]
glucuronate catabolic process to xylulose 5-phosphate	2	1.4	0.029	[33]
glycosylceramide catabolic process	2	1.4	0.029	[60]
cellular response to interleukin-6	3	2	0.029	[39,40]
cell-matrix adhesion	4	2.7	0.03	[50,57]
sodium ion transport	4	2.7	0.031	[41]
membrane fusion	3	2	0.031	
antimicrobial humoral immune response mediated by antimicrobial peptide	4	2.7	0.033	
acute-phase response	3	2	0.034	
dermatan sulfate catabolic process	2	1.4	0.036	[61]
L-ascorbic acid biosynthetic process	2	1.4	0.036	[42]
protein localization to plasma membrane	5	3.4	0.036	
regulation of actin cytoskeleton organization	4	2.7	3.60E-02	
killing of cells of other organism	3	2	3.70E-02	
innate immune response	8	5.4	3.70E-02	[43]
hematopoietic progenitor cell differentiation	4	2.7	4.20E-02	[44]
positive regulation of substrate adhesion-dependent cell spreading	3	2	4.20E-02	[50,57]
cellular response to inorganic substance	3	2	4.20E-02	
ossification	4	2.7	4.30E-02	[45]
L-cystine transport	2	1.4	4.30E-02	[46]
glycoside catabolic process	2	1.4	4.30E-02	
establishment of Sertoli cell barrier	2	1.4	4.30E-02	
membrane raft organization	2	1.4	4.30E-02	

response to inorganic substance	3	2	4.40E-02	
cellular response to transforming growth factor beta stimulus	4	2.7	4.90E-02	[62]

3.3.4 分子功能和 KEGG 通路分析

将 157 个差异蛋白（P 值<0.05，FC>1.5 或<0.67）导入 DAVID 数据库，富集到 23 个分子功能，如表 4 所示。

表 4 Fe-D0 组和 Fe-D4 组差异蛋白（P 值<0.05，FC>1.5 或<0.67）的分子功能（MF）富集分析（P 值<0.05，按照 P 值从小到大排序）

Term	Count	%	P-Value
macromolecular complex binding	21	14.2	2.00E-07
integrin binding	9	6.1	1.90E-05
calcium ion binding	18	12.2	2.90E-05
protein homodimerization activity	18	12.2	5.30E-05
sulfuric ester hydrolase activity	4	2.7	7.00E-05
protein binding	28	18.9	3.40E-04
calcium-dependent protein binding	6	4.1	8.00E-04
complement binding	3	2	1.20E-03
heparin binding	7	4.7	1.90E-03
arylsulfatase activity	3	2	2.00E-03
glucose transmembrane transporter activity	3	2	6.60E-03
cysteine-type endopeptidase inhibitor activity	4	2.7	6.70E-03
calcium-dependent phospholipid binding	4	2.7	8.30E-03
receptor binding	9	6.1	9.50E-03
extracellular matrix structural constituent	4	2.7	1.50E-02
N-acetylgalactosamine-6-sulfatase activity	2	1.4	1.50E-02
alpha-galactosidase activity	2	1.4	1.50E-02
cytoskeletal protein binding	4	2.7	1.60E-02
transmembrane transporter activity	5	3.4	1.80E-02
protease binding	5	3.4	2.00E-02
protein phosphorylated amino acid binding	2	1.4	3.00E-02
water transmembrane transporter activity	2	1.4	3.80E-02
inorganic diphosphatase activity	2	1.4	4.50E-02

将 157 个差异蛋白（P 值<0.05，FC>1.5 或<0.67）导入 DAVID 数据库，富集到 10 个 KEGG 通路。富集到的 KEGG 通路包括溶酶体、补体和凝血级联、黏着糖胺聚糖降解、肌动蛋白细胞骨架的调节、阿米巴病、疟疾、白细胞经内皮迁移、系统性红斑狼疮、代谢通路。（表 5）

溶酶体是铁代谢的主要调节剂[63]。静脉注射铁制剂诱导体内补体活化[33]。细胞内氧化铁纳米颗粒浓度高影响细胞骨架和黏着斑激酶介导的信号传导[64]。服用铁会大大增加膳食缺铁牧民对阿米巴病的易感性[65]。铁是恶性疟原虫发展的辅助因子[66]。蔗糖铁和葡萄糖酸铁对多形核白细胞(polymorphonuclear leukocyte, PMN)的跨内皮迁移有显著抑制作用[67]。许多研究已经证明了铁在免疫反应中的重要作用，并且越来越多的证据表明，在系统性红斑狼疮的慢性炎症状态下，铁稳态可能发生异常[68]。

表 5 Fe-D0 组和 Fe-D4 组差异蛋白（P 值<0.05，FC>1.5 或<0.67）的 KEGG 通路富集分析（P 值<0.05，按照 P 值从小到大排序）

Term	Count	%	P-Value	Related to Iron
Lysosome	9	6.1	4.80E-05	[63]
Complement and coagulation cascades	7	4.7	2.00E-04	[33]
Focal adhesion	9	6.1	7.90E-04	[64]
Glycosaminoglycan degradation	4	2.7	9.20E-04	
Regulation of actin cytoskeleton	9	6.1	2.00E-03	[64]
Amoebiasis	5	3.4	1.50E-02	[65]
Malaria	4	2.7	1.70E-02	[66]
Leukocyte transendothelial migration	5	3.4	2.80E-02	[67]
Systemic lupus erythematosus	5	3.4	2.90E-02	[68]
Metabolic pathways	24	16	3.50E-02	

3.4 单只大鼠自身前后对照

3.4.1 差异蛋白筛选情况

尿液蛋白质组能够很灵敏地反映机体状态的变化，也会在一定程度上受到遗传因素[69]、年龄[70-72]、性别[73,74]、民族[75]、地域[76]、运动[77,78]、饮食习惯、精神状态、昼夜节律、用药情况[79,80]等环境因素的影响，表现出同一个体和个体之间的差异性[81,82]。动物模型易于控制变量，可以减少人类尿液样本中由于无关变量产生的变化，但是，即便是同种动物的不同个体之间也存在一些差异。因此，本研究使用了自身前后对照的分析方法，能够减少个体差异性的影响，有助于识别潜藏的重要信息。

自身前后比较的具体分析方法如下：将缺失值替换成 0，将每只大鼠灌胃前样本（D0）的三针重复与灌胃第 4 天样本（D4）的三针重复进行双尾、成对比较，筛选差异蛋白条件是：T 检验分析的 P 值<0.05，倍数变化 Fold change (FC)>1.5 或<0.67。

筛选结果如下：1 号大鼠筛选到 194 个差异蛋白，2 号大鼠筛选到 368 个差异蛋白，3 号大鼠筛选到 520 个差异蛋白，4 号大鼠筛选到 230 个差异蛋白，5 号大鼠筛选到 148 个差异蛋白。

3.4.2 共有生物学过程、分子功能、通路分析

利用 DAVID 数据库将五只大鼠的差异蛋白分别进行功能注释，筛选条件为 p<0.05。并用韦恩图分析 5 只大鼠生物学过程、分子功能、通路的重叠情况。

1 号大鼠富集到 126 个生物学过程；2 号大鼠富集到 163 个生物学过程；3 号大鼠富集到 212 个生物学过程;4 号大鼠富集到 167 个生物学过程;5 号大鼠富集到 77 个生物学过程。

在 5 只大鼠（占实验组总数的 100%）中共有的生物学过程有 3 个，包括碳水化合物代谢过程、老化、细胞-基质粘附。文献显示，铁代谢失调和衰老[32]、葡萄糖代谢途径是相互关联的[33]，铁死亡与细胞粘附等多种信号通路有关[50,57]。

16 个生物学过程在 4 只大鼠（占实验组总数的 80%）中共有。用表格展示了这些生物学过程以及与铁相关的文献。此外，对铁离子的反应，血红素对 eIF2 α 磷酸化的调节等生物学过程在 3 只大鼠（占实验组总数的 60%）中共有。

表 6 在 4 只或 5 只大鼠中共有的生物学过程（BP）（DAVID 数据库 GO 分析）

Rats	Biological Process(BP)	Related to Iron

1 2 3 4 5	carbohydrate metabolic process	[33]
1 2 3 4 5	aging	[32]
1 2 3 4 5	cell-matrix adhesion	[83]
1 2 3 4	negative regulation of cysteine-type endopeptidase activity	[46]
1 2 3 4	negative regulation of endopeptidase activity	[56]
1 2 3 4	positive regulation of fibroblast proliferation	[50]
1 2 3 4	lipid metabolic process	[84]
1 2 3 4	cell adhesion mediated by integrin	[50,57]
1 2 3 4	response to estrogen	[34,35]
1 2 3 4	glomerular filtration	[85,86]
1 2 3 5	phagocytosis, engulfment	[87]
1 3 4 5	positive regulation of cell migration	[88]
1 3 4 5	positive regulation of ERK1 and ERK2 cascade	[89]
1 3 4 5	cellular response to lipopolysaccharide	[90,91]
1 3 4 5	positive regulation of protein kinase B signaling	[92]
2 3 4 5	zymogen activation	[56]
2 3 4 5	regulation of systemic arterial blood pressure	[93]
2 3 4 5	complement activation, classical pathway	[47]
2 3 4 5	glutathione metabolic process	[94]

1 号大鼠富集到 35 个分子功能；2 号大鼠富集到 67 个分子功能；3 号大鼠富集到 75 个分子功能；4 号大鼠富集到 61 个分子功能；5 号大鼠富集到 31 个分子功能。

在 5 只大鼠（占实验组总数的 100%）中共有的分子功能有 3 个，包括蛋白质结合、大分子络合物结合、钙离子结合。11 个分子功能在 4 只大鼠（占实验组总数的 80%）中共有，其中包括血红蛋白结合（hemoglobin beta binding）。铁在体内是血红蛋白合成的重要组成部分，对于氧的运输和细胞呼吸至关重要。

表 7 在 4 只或 5 只大鼠中共有的分子功能（MF）（DAVID 数据库 GO 分析）

Rats	Molecular function (MF)
1 2 3 4 5	protein binding
1 2 3 4 5	macromolecular complex binding
1 2 3 4 5	calcium ion binding
1 2 3 4	proton-transporting ATPase activity, rotational mechanism
1 2 3 4	hydrolase activity
1 2 3 4	protease binding
1 2 3 4	cysteine-type endopeptidase inhibitor activity
1 2 3 4	serine-type endopeptidase inhibitor activity
1 2 3 4	phosphatidylserine binding
1 2 4 5	hemoglobin beta binding
1 3 4 5	endopeptidase inhibitor activity
2 3 4 5	peptidase activity
2 3 4 5	receptor binding
2 3 4 5	serine-type endopeptidase activity

利用 DAVID 网站进行京都基因和基因组百科全书数据库（Kyoto encyclopedia of genes and genomes，KEGG）通路富集分析。1 号大鼠富集到 30 个 KEGG 通路；2 号大鼠富集到 41 个 KEGG 通路；3 号大鼠富集到 49 个 KEGG 通路；4 号大鼠富集到 35 个 KEGG 通路；5 号大鼠富集到 10 个 KEGG 通路。

在 5 只大鼠（占实验组总数的 100%）中共有的 KEGG 通路有溶酶体、吞噬体。5 个 KEGG 通路在 4 只大鼠（占实验组总数的 80%）中共有，包括疟疾、内吞作用、非洲锥虫病、金黄色葡萄球菌感染、鞘脂类代谢。提示通路与铁关联的文献已列于表格中。

表 8 在 4 只或 5 只大鼠中共有的 KEGG 通路（DAVID 数据库 GO 分析）

Rats	Kyoto encyclopedia of genes and genomes(KEGG) pathway	Related to Iron
1 2 3 4 5	Lysosome	[63]
1 2 3 4 5	Phagosome	[95]
1 2 4 5	Malaria	[66]
1 2 4 5	Endocytosis	[96]
1 3 4 5	African trypanosomiasis	[97]
1 3 4 5	Staphylococcus aureus infection	[58]
2 3 4 5	Sphingolipid metabolism	[98]

3.4.3 多只大鼠共同上调或下调的差异蛋白分析

把每只大鼠前后比较得到的差异蛋白按照 FC 分成上调、下调。相较于灌胃前样本（D0），5 只大鼠灌胃后样本（D4）中分别鉴定到 129 个、309 个、425 个、148 个、69 个上调的差异蛋白（FC>1.5,P<0.05）。5 只大鼠灌胃后分别鉴定到 65 个、59 个、95 个、82 个、79 个下调的差异蛋白（FC<0.67,P<0.05）。用韦恩图展示 5 只大鼠灌胃前后鉴定到差异蛋白重叠情况，如图 4、图 5 所示。差异蛋白名称和重叠情况列在补充表格中。

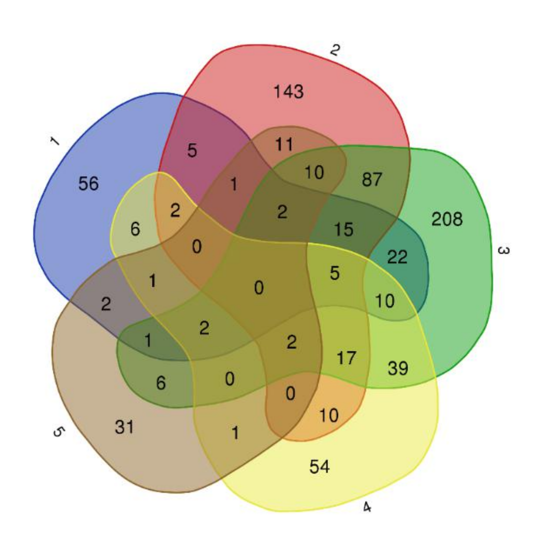


图 4 5 只大鼠自身前后对照产生的上调差异蛋白（FC>1.5,P<0.05）韦恩图

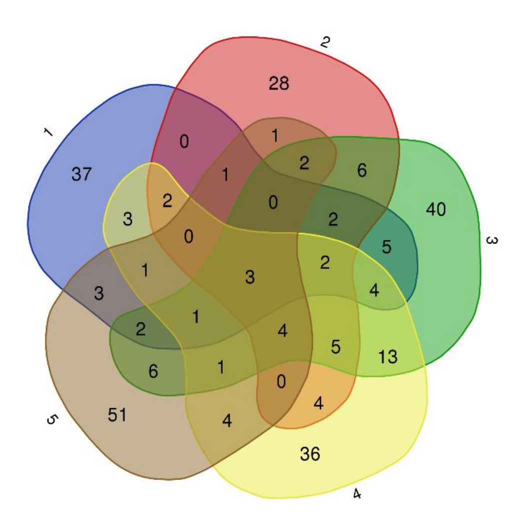


图 5 5 只大鼠自身前后对照产生的下调差异蛋白（FC<0.67,P<0.05）韦恩图

对于其中在 4 只或 5 只大鼠中共同上调或下调的差异蛋白，进行详细的搜索和分析，如表 9 所示。

S100 钙结合蛋白 A7（S100 calcium binding protein A7 like 2,S100A7l2）、前列腺类固醇结合蛋白 C1（Prostatic steroid-binding protein C1）、胱抑素相关蛋白（Cystatin-related protein 1）在 5 只大鼠中共同下调。孕期缺铁饮食大鼠的子代大脑中 S100 钙结合蛋白的表达量降低[6]。前列腺上皮细胞合成铁调素，并且在前列腺癌细胞和组织中铁调素的合成和分泌显着增加[99]。胱抑素 C 和血清铁蛋白成正相关[100]。

精胺结合蛋白（Spermine binding protein）、胱抑素相关蛋白 2（Cystatin-related protein 2）、Cullin 1、衰变加速因子 1（Decay accelarating factor 1）、前列腺钾化素-6（Prostatic glandular kallikrein-6）、颌下腺小钾素-9（Submandibular glandular kallikrein-9）等 7 种蛋白质在 4 只大鼠中下调。查阅文献发现，多种蛋白质（或其家族）与铁代谢或铁蛋白有关，详见表 9。

11 种蛋白质在 4 只大鼠中上调，其中包括铁调素（Hepcidin），铁调素作为信号分子参与维持铁稳态。H-2 II 类组织相容性抗原γ链（H-2 class II histocompatibility antigen gamma chain）、中性和碱性氨基酸转运蛋白 rBAT（Neutral and basic amino acid transport protein rBAT）、溶质载体家族 22 成员 12（Solute carrier family 22 member 12）、酰基辅酶 A 合成酶短链家族成员 3（Acyl-CoA synthetase short-chain family member 3）、谷氨酸-半胱氨酸连接酶催化亚基（Glutamate-cysteine ligase catalytic subunit）、β-半乳糖苷酶（Beta-galactosidase）等多种蛋白质也被搜索到与铁代谢或铁蛋白有关。

表 9 在 4 只或 5 只大鼠中共同上调或下调的差异蛋白

Protein Accessions	Gene Names	Trend	Related to Iron
D3ZFC6	Itih4	1⬆2⬆3⬆4⬆	[101,102]
F1M8K0	Dag1	1⬆2⬆3⬆4⬆	
E9PT83	Cenpf	1⬆2⬆3⬆4⬆	
P51635	Akr1a1 Alr	1⬆2⬆3⬆4⬆	
P10247	Cd74	1⬆2⬆3⬆4⬆	[22]
Q64319	Slc3a1 Nbat	1⬆2⬆3⬆5⬆	[24,25]
Q3ZAV1	Slc22a12 Urat1	1⬆2⬆3⬆5⬆	[103]
Q99MH3	Hamp Hepc	1⬆3⬆4⬆5⬆	[104]
A0A0G2K047	Acss3	1⬆3⬆4⬆5⬆	[105]

P19468	Gclc Glclc	2↑3↑4↑5↑	[106]
D3ZUM4	Glb1	2↑3↑4↑5↑	[19]
D3Z9U8	S100a7l2 RGD1562234	1↓2↓3↓4↓5↓	[6]
P02782	Psbpc1 Scgb1d2	1↓2↓3↓4↓5↓	[99]
P22282	Andpro Crp1	1↓2↓3↓4↓5↓	[100]
A0A0G2K176	Sbp Zg16b	1↓2↓3↓4↓	[107]
Z4YNX7	P22k15	1↓2↓3↓4↓	[100]
B1WBY1	Cul1	1↓3↓4↓5↓	[108]
P22283	Crp2 P22k15	2↓3↓4↓5↓	[100]
A0A0G2QC50	Cd55 Daf1	2↓3↓4↓5↓	[109]
P36374	Klk6 Klk-8 Klk8	2↓3↓4↓5↓	[110]
P07647	Klk9 Klk-9 Klks3	2↓3↓4↓5↓	[110]

3.4.4 多只大鼠共同上调或下调的差异蛋白的富集功能分析

对于在 3 只、4 只或 5 只大鼠中共同上调或下调的差异蛋白进行功能注释，对于这些差异蛋白富集到的生物学过程（表 10）、分子功能（图 6）、KEGG 通路（表 11）进行分析。

共富集到 44 个生物学过程，并对富集到的生物学过程与铁的相关性进行了检索，相关文献详见表 10。

其中，有 15 个生物学过程与成组分析的结果重合，包括酶原活化、成纤维细胞增殖的正调控、细胞基质粘附、老化、整合素介导的细胞粘附、抗菌肽介导的抗菌体液免疫反应、对雌激素的反应、急性期反应、肽基-酪氨酸磷酸化的正调控、对乙醇的反应、细胞凋亡过程的负调控、L-胱氨酸转运、糖苷分解代谢过程、碳水化合物代谢过程、肾上腺近端肾小管发育。

此外，还富集到了谷胱甘肽代谢过程、全身动脉血压的调节、血红素对 eIF2α 磷酸化的调控等生物学过程。许多生物学过程都与铁的生物学功能有关。

表 10 3 只或以上大鼠中共同上调或下调的蛋白质的生物学过程（BP）富集分析（DAVID 数据库 GO 分析）

Biological Process(BP)	Count	%	P-Value	Related to Iron
glutathione metabolic process	5	5.4	0.0002	[94]
regulation of systemic arterial blood pressure	4	4.3	0.00032	[93]
zymogen activation	4	4.3	0.00047	[56]
amino acid transmembrane transport	4	4.3	0.00055	[50, 57]
positive regulation of fibroblast proliferation	5	5.4	0.0006	[50]
cell-matrix adhesion	5	5.4	0.00083	[50, 57]
aging	8	8.6	0.00094	[32]
negative regulation of cysteine-type endopeptidase activity	3	3.2	0.0015	[100]
binding of sperm to zona pellucida	4	4.3	0.0015	[111]
response to hormone	5	5.4	0.0016	[112]
positive regulation of cell migration	7	7.5	0.0018	[88]
L-glutamate transport	3	3.2	0.0036	[113]
negative regulation of endopeptidase activity	4	4.3	0.0039	
phagocytosis	4	4.3	0.0041	[87]
amino acid transport	3	3.2	0.006	[50, 57]

cell adhesion mediated by integrin	3	3.2	0.011	[114]
antimicrobial humoral immune response mediated by antimicrobial peptide	4	4.3	0.013	[115]
response to estrogen	4	4.3	0.016	[34,35]
acute-phase response	3	3.2	0.017	[61]
positive regulation of peptidyl-tyrosine phosphorylation	4	4.3	0.017	
negative regulation of cell projection organization	2	2.2	0.018	
positive regulation of lysosomal protein catabolic process	2	2.2	0.018	[116]
regulation of eIF2 alpha phosphorylation by heme	2	2.2	0.018	[117]
regulation of acrosome reaction	2	2.2	0.022	[118]
response to ethanol	5	5.4	0.023	[54,55]
negative regulation of apoptotic process	8	8.6	0.023	[38]
cellular response to mechanical stimulus	4	4.3	0.024	
positive regulation of ERK1 and ERK2 cascade	5	5.4	0.025	[89]
L-cystine transport	2	2.2	0.026	[46]
glycoside catabolic process	2	2.2	0.026	
carbohydrate metabolic process	4	4.3	0.027	[49]
positive regulation of protein localization to plasma membrane	3	3.2	0.028	
wound healing	4	4.3	0.03	[119]
cell-cell adhesion mediated by integrin	2	2.2	0.031	[50,57,114]
lipid metabolic process	4	4.3	0.034	[84]
cellular response to mercury ion	2	2.2	0.035	[120]
positive regulation of cell death	3	3.2	0.039	[121]
nitric oxide transport	2	2.2	0.039	[122]
metanephric proximal tubule development	2	2.2	0.044	[48]
renal absorption	2	2.2	0.044	[48]
aspartate transport	2	2.2	0.044	
cell migration	5	5.4	0.048	[123]
glutathione biosynthetic process	2	2.2	0.048	[94]
hyperosmotic response	2	2.2	0.048	

富集到 17 个分子功能，其中包括血红蛋白结合。半胱氨酸型内肽酶抑制剂活性、蛋白酶结合、大分子复合物结合、受体结合、钙离子结合、整合素结合、芳基硫酸酯酶活性等 7 个分子功能与成组分析的结果重合。

富集到 10 个 KEGG 通路，其中有 4 个与成组分析的结果重合，包括溶酶体、糖胺聚糖降解、肌动蛋白细胞骨架的调节、疟疾。对富集到的 KEGG 通路与铁的相关性进行了检索，相关文献详见表 11。

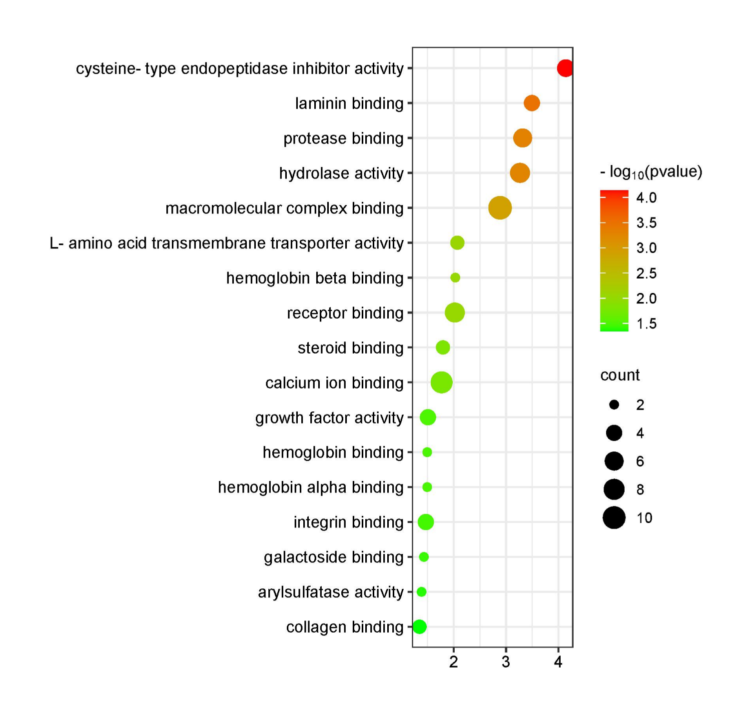


图 6 3 只或以上大鼠中共同上调或下调的蛋白质的分子功能（MF）富集分析（DAVID 数据库 GO 分析）

表 11 3 只或以上大鼠中共同上调或下调的蛋白质的 KEGG 通路富集分析（DAVID 数据库 GO 分析）

KEGG Pathway	Count	%	P-Value	Related to Iron
Lysosome	7	7.5	0.000085	[63]
Sphingolipid metabolism	4	4.3	0.0035	[98]
Other glycan degradation	3	3.2	0.0043	[124]
Glycosaminoglycan degradation	3	3.2	0.0053	[125]
Regulation of actin cytoskeleton	6	6.5	0.0089	[64]
Gap junction	4	4.3	0.012	[126]
Phagosome	5	5.4	0.019	[95]
African trypanosomiasis	3	3.2	0.02	[97]
Cholesterol metabolism	3	3.2	0.031	[84]
Malaria	3	3.2	0.036	[66]

4 展望

铁过载通常被定义为机体内铁元素积累过多，超出正常代谢需要的范围。这种情况可能由多种原因引起，例如长期补充铁剂、遗传性疾病（如遗传性血色病）、慢性炎症状态等。近年来，有关铁过载的发生及其负性效应已引起关注。铁过载在世界各地都有发生，特别在经济较为发达地区，严重影响人类(尤其儿童)的健康与生命安全。铁过载影响脂质过氧化，营养代谢，与心血管疾病发生、发展密切相关。铁过载对生物体健康带来的影响是多方面的，包括但不限于细胞内氧化应激加剧、组织损伤、器官功能受损，可导致严重的心血管疾病和神经系统疾病。

本研究中，大鼠灌胃多糖铁复合物的剂量为 28mg/kg·d（按铁计），相当于成年人预防贫血的剂量。根据文献调研，本研究使用的多糖铁复合物浓度如果应用于建立铁过载模型，

需要灌胃 4 周以上[127]。本研究对大鼠灌胃多糖铁复合物（28mg/kg·d 铁）4 天，旨在探究短期多糖铁复合物灌胃对机体的整体影响。本研究有望为铁代谢紊乱相关疾病（比如铁缺乏导致的贫血和铁过载导致的心血管疾病等）的预防、诊断、治疗及监测提供一些线索，填补尿液蛋白质组在铁代谢领域的空白。

本研究采用了自身前后比较和成组比较两种分析方法，这为我们提供了更加全面和可靠的数据验证。前后比较方法的应用减少了个体差异对实验结果的影响，提高了实验的稳定性和可重复性，对结果的可信度具有重要意义。两种分析方法得到的结果互为验证，说明尿液蛋白质组能够反映短期摄入多糖铁复合物对机体的影响，使得结果更加可信。

研究结果说明，短期摄入多糖铁复合物后，大鼠的尿液蛋白质组可以显示出与铁相关的蛋白质和生物学功能的变化。短期补充多糖铁复合物会对机体产生影响，而尿液蛋白质组能够全面、系统地反映机体的整体变化。本研究从尿液蛋白质组学的角度为深入理解铁元素在生物体内的代谢过程、作用机制、生物学功能提供了线索，同时为未来相关研究提供了新的研究视角和方法学启示，这对于铁代谢紊乱相关疾病的预防、诊断、治疗及监测有着潜在的重要意义。

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